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(54) Title: METHOD AND COMPOSITION FOR IMMUNIZATION USING MIXED POOLS OF MUTATED NUCLEIC ACIDS OR PEPTIDES

(57) Abstract: An immune response to a target antigen is induced in a subject be administering to the subject a vaccine composition in an amount sufficient to induce an immune response to the target antigen. The vaccine composition contains a mixed pool of a plurality of DNA or RNA species encoding a plurality of mutant forms of the target antigen which are expressed of the mixed pool of nucleic acids. The vaccine compositions of the invention are administered to a subject, for example a human subject, to induce an immune response to the target antigen.

# METHOD AND COMPOSITION FOR IMMUNIZATION USING MIXED POOLS OF MUTATED NUCLEIC ACIDS OR PEPTIDES

## DESCRIPTION

The research described in this application was supported by a grant from the National Cancer Institute, Award No. R0156821. The United States may have certain rights in this invention.

## Background of the Invention

This application relates to a method for immunization which makes use of mixed pools of mutated nucleic acids (DNA or RNA) encoding variants of a target antigen as a nucleic acid vaccine or expressed peptides derived from such pools to stimulate an immune response to the target antigen.

The immune system provides a sophisticated and multi-faceted defense against antigens which are recognized as foreign. Antigens which are recognized, and which therefore stimulate an immune response, are referred to as immunogenic. However, not all antigens are immunogenic, and there are many instances of disease that the immune system deals with poorly, if at all. These include most cancers and infectious organisms such as human immunodeficiency virus-1, Mycobacterium tuberculosis, Borrelia burgdorferi (the causative organism of Lyme disease), Epstein Barr virus, papilloma virus, hepatitis viruses and cytomegalovirus (CMV) to which the immune system fails to mount an effective response. This may be due to tolerance to self-antigens (i.e. the body does not recognize a cancer cell as foreign) or because the antigen is an inherently weak immunogen. Regardless of the cause, however, these factors make the product of vaccines targeting these conditions both desirable and difficult.

Various approaches have been taken to try to overcome the inherent non-immunogenicity of cancer and other antigens. These approaches have included the identification of more immunogenic variants of the antigens of interest through modification of the epitope structure or through modification of MHC-binding regions, as well as the addition of adjuvants to enhance antigenicity. The general concept behind these prior approaches is the identification of a single species of antigen with improved

immunogenicity. This can be based on structural information at the crystallographic level, information which is available for only a very limited number of human or animal MHC molecules, or extensive trial and error testing. The present invention represents a departure from this traditional approach.

Summary of the Invention

The present invention provides a method for inducing an immune response to a target antigen in a subject comprising administering to the subject a vaccine composition in an amount sufficient to induce an immune response to the target antigen. The vaccine composition comprises a mixed pool of a plurality of DNA or RNA species encoding a plurality of mutant forms of the target antigen which are expressed by the subject, or a mixture of peptides derived from pre-administration expression of the mixed pool of nucleic acids. The vaccine compositions of the invention are administered to a subject, for example a human subject, to induce an immune response to the target antigen.

Brief Description of the Drawings

Fig. 1 shows the number of tumor-free surviving mice immunized with nucleic acid vaccine derived from murine gp75 using various protocols;

Fig. 2 shows the number of tumor-free surviving mice immunized with nucleic acid vaccine derived from murine TRP-2 using various protocols; and

Fig. 3 shows the quantitative survey of autoimmune depigmentation in mice immunized with nucleic acid vaccine derived from murine gp75 using various protocols.

Detailed Description of the Invention

The present invention provides a method and composition for inducing an immune response to a target antigen. In accordance with the invention, nucleic acid vaccine compositions are administered to a subject in a manner which results in expression of the nucleic acid and recognition of the resulting proteins or peptides by the immune system.

The target antigen against which the invention induces an immune response may be any antigen for which a therapeutic benefit is derived as a result of the induction of an

immune response, including antigens associated with pathogenic microorganisms and antigens associated with cancers. The invention is particularly applicable for inducing an immune response to inherently non-immunogenic or poorly immunogenic antigens. Specific, non-limiting examples of target antigens include gp75/TRP-1, TRP-2, tyrosinase, gp100/pMel17 on melanoma; prostate specific membrane antigen, prostate specific antigen and prostate stem cell antigen on prostate cancers; HER2/neu and the mucin MUC1 on breast cancers; CD19 and CD20 on malignancies of B lymphocyte origin; MAGE, BAGE and GAGE, NY-ESO-1 and other "cancer-testes" antigens on a variety of cancer types; gene products from the human immunodeficiency virus-1; angiogenic factors (such as VEGF, bFGF, angiopoietins, their cognate cell surface receptors, and ELR C-X-C chemokines); tumor suppressor genes such as p53; dipeptidyl peptidase IV and fibroblast activation protein-1.

As used in the specification and claims of this application, the phrase "inducing an immune response" refers to both the stimulation of a new immune response or to the enhancement of a pre-existing immune response to a target antigen. The immune response may be a cytolytic T-cell mediated cellular immune response or a B-cell mediated humoral response, or some combination thereof.

The term "subject" refers to the living organism being treated to induce an immune response. The subject will generally be mammalian or avian. Preferred "subjects" are human patients.

In the method of the invention, an immune response to a target antigen is induced in a subject comprising administering to the subject a vaccine composition in an amount sufficient to induce an immune response to the target antigen, wherein the vaccine composition comprises a plurality of vaccine molecule species corresponding to a plurality of mutant forms of the target antigen. As used herein, the term "vaccine molecule species" refers to either nucleic acids (DNA or RNA) or to peptides. The term "corresponding" encompasses both mutant forms of the target antigen per se (i.e, peptide vaccine molecule species) and nucleic acid vaccine molecule species encoding the mutant forms of the target antigen.

In a first embodiment of the invention, the vaccine compositions of the invention comprise a mixture of mutated nucleic acids which encode mutant variants of the target

antigen. Unlike prior methods which focus on using single, carefully selected mutant antigens, the present invention intentionally uses a mixture of different species of mutant nucleic acid all derived from a starting nucleic acid encoding the target antigen. To prepare such a mixture, one starts with a nucleic acid sequence encoding the target antigen. This sequence may be in the form of PCR amplicon, or it may be incorporated in a vector system to facilitate its reproduction in an appropriate host. The sequence may be cDNA encoding the entire antigen or it may be a partial sequence encoding only a portion of the antigen. Although there is no absolute minimum size, partial sequences used will preferably be at least 24 bases, encoding 8 amino acids. This "starting nucleic acid sequence" is used as the starting material for generating the vaccine compositions of the invention.

The starting nucleic acid sequence may be an accepted "wild-type" sequence derived from a normal source. In this regard, it will be appreciated that polymorphic sequences may have a multiplicity of "normal" or "wild-type" sequences, and that it is not critical which of these sequences are used as the starting sequence. The starting nucleic acid sequence may also be a mutant sequence (i.e. a sequence which differs from the established norm.) The starting nucleic acid sequence may also be (but does not have to be) derived from the subject. Thus, for example, in the latter case, a subject's own cancer cells could be used as a source for the starting nucleic acid sequence.

Mutations, which can be insertions, deletions, translations, or inversions of one or more bases, can be introduced into the starting nucleic acid sequence using any of various known techniques. For example, random mutations can be introduced into the starting nucleic acid

sequences using error-prone PCR as described in Cadwell et al. in PCR Methods and Applications 2:28-33 (1992) and PCR Methods and Applications 3:5136-5140 (1994). Mutations can also be introduced into the starting nucleic acid sequence by expressing the starting nucleic acid sequence in bacteria that are prone to mutations (for example Stratagene's XL 1-RED competent cells) or by exposing the starting nucleic acid to mutagenic principles such as chemicals, x-rays or ultraviolet radiation.

The result of these methods is a library of nucleic acid made up of many copies of mutated nucleic acid, with most individual nucleic acid molecules containing unique

combinations of mutations. Aliquots of this library are then subcloned into an expression vector to make a "pool" of mutant nucleic acid for use in a vaccine. Such a pool will suitably contain at least 50, preferably at least 400, or even 2000-3000 different mutant nucleic acid species. If the starting sequence is a complex population of sequences (such as a cDNA library), the number of clones is driven by the sensitivity of the immunization protocol. This pool is used to form the vaccine compositions of the invention.

The nucleic acids in the pool may be either DNA or RNA since both are known to useful in vaccine compositions. (See, for example, US Patents Nos. 5,580,859 and 5,589,466, Qiu et al., "Gene gun delivery of mRNA in situ results in efficient transgene expression and genetic immunization." *Gene Ther.* 3(3):262-8 (1996); Garrity RR., "Targeted immune design using RNA immunization"*Ann N Y Acad Sci.*894:124-9 (1999); Zhou et al., "RNA melanoma vaccine: induction of antitumor immunity by human glycoprotein 100 mRNA immunization", *Hum Gene Ther.* 10(16):2719-24 (1999); Giraud et al., "Generation of monoclonal antibodies to native human immunodeficiency virus type 1 envelope glycoprotein by immunization of mice with naked RNA", *J Virol Methods.* 79(1):75-84 (1999); Dalemans et al., "Protection against homologous influenza challenge by genetic immunization with SFV-RNA encoding Flu-HA", *Ann N Y Acad Sci.* 772:255-6 (1995); Heiser et al., "Human dendritic cells transfected with RNA encoding prostate-specific antigen stimulate prostate-specific CTL responses In vitro", *J Immunol.* 164(10):5508-14 (2000); Boczkowski et al., "Induction of tumor immunity and cytotoxic T lymphocyte responses using dendritic cells transfected with messenger RNA amplified from tumor cells", *Cancer Res.*60(4):1028-34 (2000); Nair et al., "Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA", *Nat Biotechnol.* 16(4):364-9 (1998); Ashley et al., "Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce antitumor immunity against central nervous system tumors", *J Exp Med.* 186(7):1177-82 (1997), all of which are incorporated herein by reference). Where the pool is DNA, and the desired vaccine contains RNA, transcription can be carried out using alpha viruses or in vitro transcription systems. It will be apparent that no individual pool will contain all of the possible mutant forms which could be made for a given target antigen. Indeed, a given pool need not even contain all of the mutant nucleic acid species formed in a given

library. As shown in the examples below, however, the selection of specific mutant forms is not required and all five pools with mutant gp75 DNA acid were effective in stimulating an antitumorigenic immune response. Furthermore, because of the comparative ease of production, several alternative pools can be easily available for use if a vaccine based on a first pool were to prove ineffective.

Once the pool of mutant nucleic acid is created, it is formulated into a vaccine composition for administration to the subject. As discussed below, one suitable mode of administration is subcutaneous injection of particles coated with the nucleic acid mixture using a GENE GUN. Thus, one embodiment of the vaccine composition comprises carrier particles coated with the pool of nucleic acid, i.e. with a mixture comprising a plurality of nucleic acid species encoding a plurality of mutant forms of the target antigen. The expressed mutant proteins or peptides are immunogenic and stimulate an immune response to the target antigen, even in the case where the target antigen is inherently non-immunogenic or only weakly immunogenic in the subject. The carrier particles used in this composition may be any of various types of particles known for use in this purpose, including without limitation gold, clay and tungsten. The particles suitably are from 0.5 to 2 microns in diameter to facilitate transdermal injection.

Other delivery systems which can be used to administer the nucleic acid vaccine compositions of the invention include the pressure delivery systems, for instance the BIOJECT system which delivers vaccines using carbon dioxide pressure cartridges. In this case, particles are not required, but can be used. The vaccine compositions can also be administered without a particle carrier using non-pressurized systems, for example syringe needles. Administration could also be accomplished using a mucosal route (e.g, a nasal spray). The pool of mutated DNA may also be incorporated into a viral vector, which is then associated with particles for adminstration by the routes described above.

The vaccine composition above may be administered in a liquid carrier by subcutaneous injection. For use in a Gene Gun, however, the composition is suitably packaged into therapeutic administration units, sometimes referred to as "bullets". This is accomplished by drawing the composition into the lumen, a thin hollow tube, and then cutting the tube into lengths containing about 1 μg of nucleic acid.

In a second embodiment of the invention, the vaccine is a peptide vaccine created by expressing the pool of nucleic acids prior to administration. Expression is suitably carried out in host cells which may be bacterial or eukaryotic (for example, yeast, insect or mammalian). For such expression, the pool of nucleic acids are incorporated into an expression vector compatible with the host cells and then introduced into the host cells for expression without specific selection steps. Such expression systems are well known in the art.

The peptide vaccine compositions of the invention are different from known vaccine compositions, such as flu vaccines, which may contain mixtures of peptide antigens, because such mixtures are mixtures of several target antigens themselves rather than of mutant forms of one target antigen. Thus, for example in the case of a flu vaccine, the peptide antigens are selected to match the known epitopes of various strains of influenza.

The peptide vaccine of the invention can be administered using methods known in the art, including without limitation by intravenous, intramuscular and subcutaneous injection

and by transdermal or intranasal administration. The determination of the appropriate amount of peptide to vaccine to administer to arrive at the desired immune response is a routine matter within the ordinary skill in the art.

The invention will now be further described with reference to the following, non-limiting examples.

Example 1

To prepare mutant DNA, full length murine tyrosine-related protein 2 (mTRP-2) and murine gp75 were randomly mutated by PCR using the protocol of Cadwell et al., *supra*. Briefly, 20 ng of non-mutated plasmid encoding either protein served as a template for PCR. Mutagenic PCR was performed in Boehringer Mannheim's 1X PCR buffer supplemented to contain 7 mM MgCl2, 0.5 mM MnCl2, 0.2 mM dATP and dGTP, and 1 mM dCTP and dTTP. 30 PCR cycles were performed with primers pairs specific for each coding sequence (mTRP-2 primers, upstream: AAGGCGGCGCATGGGCCTTGTGGGATG (Seq. ID. No. 1), downstream: ATGCGGCCGCTAGGCTTCCTCCGTGTA (Seq. ID. No. 2); mgp75 primers,

upstream: TTGCGGCCGCCATGAAATCTTACAACGTG (Seq. ID. No. 3), downstream: CGGAATTCTCAGACCATGGAGTGGTTA (Seq. ID. No. 4)) and Taq polymerase from Boehringer Mannheim. The primers contained unique restriction sites for subsequent subcloning. Nonmutagenic control reactions were performed with the same template and primers using Stratagene's Pfu Turbo DNA polymerase in the corresponding buffer.

To generate pools of mutants, mutated and unmutated PCR products were subcloned into the CMV-based plasmid expression vector WRGBEN. Ross et al., Clin. Can. Res. 3: 2191-2196 (1997). The clones deriving from mutagenic PCR were plated and grown, so as to obtain pools of approximately 2,500 clones. The plasmid DNA of these clones was purified using QIAGEN 500 maxiprep columns in batches of variant plasmids containing random mutations. Each batch is referred to as a pool.

To assess the efficiency of PCR mutagenesis, four mutagenized clones of both mgp75 and mTRP-2 were sequenced over the first 600 bp of the coding sequence. Tables 1 and 2 list the observed mutations by type and extrapolate the numbers to the full-length coding sequences for mgp75 mutagenesis and mTRP-2 mutagenesis, respectively. The overall mutation frequency observed after 30 PCR cycles is 1.2 per 100 bp for mgp75, and 0.8 per 100 bp for mTRP-2.

Table 1

| Clone # / Mutation Type | 1 | 2 | 3 | 4 | overall | extrapolated mean per full length mgp75 |
|---|---|---|---|---|---|---|
| Total mutations | 6 | 9 | 6 | 8 | 29 | 19 |
| transitions | 2 | 6 | 1 | 5 | 14 | 9 |
| transversions | 4 | 3 | 5 | 3 | 15 | 10 |
| missense | 2 | 3 | 4 | 5 | 14 | 9 |
| nonsense | 1 | 0 | 0 | 1 | 2 | 1 |
| silent | 3 | 6 | 2 | 2 | 13 | 9 |

| Table 2 | | | | | | |
|---|---|---|---|---|---|---|
| Clone # / Mutation Type | 1 | 2 | 3 | 4 | overall | extrapolated mean per full length mTRP-2 |
| Total mutations | 3 | 3 | 4 | 10 | 20 | 13 |
| transitions | 1 | 2 | 1 | 5 | 9 | 6 |
| transversions | 2 | 1 | 3 | 5 | 11 | 7 |
| missense | 1 | 3 | 3 | 4 | 11 | 7 |
| nonsense | 0 | 0 | 0 | 1 | 1 | 1 |
| silent | 2 | 0 | 1 | 5 | 8 | 5 |

Example 2

DNA from the plasmid pools was coated onto 1 m gold particles (BioRad) at a ratio of 100 g DNA for 50 mg of gold. The gold/DNA precipitate was deposited in plastic tubing which was cut into "bullets", each representing 1 g of DNA. Bullets were loaded in a helium-pressure POWDER-JECT gene gun for genetic immunization of mice by delivery of the DNA-coated gold to the epidermis.

Example 3

C57BL/6 mice were depilated and immunized four times at intervals of 8-10 days, receiving 4 g of DNA at each immunization. The DNA was delivered to the abdomen at three sites and the base of the tail. There were 5 mice per group. Control groups received empty vector DNA, or the unmutated murine sequence or the human ortholog of either protein in the WRGBEN vector. Seven days after the last immunization, mice received 105 B16F10LM3 syngeneic melanoma cells in 50 l of serum-free RPMI1640 medium, injected intradermally in the right flank. Tumor growth and coat color change (depigmentation) were measured every 2-3 days for 40 days minimum. Depigmentation was quantified by densitometric analysis of digital images of the immunized animals. Mice were sacrificed upon ulceration of the tumor or when it reached a diameter of 20 mm.

Tumor growth was monitored in untreated control animals, in animals injected with xenogeneic (human) gp75, in animals injected with wild-type syngeneic (mouse) gp75 and in animals injected with a mutant mgp75 pool in accordance with the invention. The results are

summarized in Fig. 1. Tumor growth was also monitored in control animals injected with empty vector, in animals injected with xenogeneic (human) TRP-2, in animals injected with wild-type syngeneic (mouse) TRP-2 and in animals injected with a mutant mTRP-2 pool in accordance with the invention. The results are summarized in Fig. 2. The fraction of tumor free mice is shown in each figure. As this data reflects, the pools of mutagenized mgp75 or mTRP-2 conferred significant protection against tumor challenge, to an extent superior to that observed with any of the controls. In addition, all mice which received one of the mutagenized pools displayed autoimmunity, as manifested by depigmentation. (see. Fig. 3) No depigmentation was observed in mice immunized with non-mutagenized syngeneic DNA. In mice immunized with xenogeneic human antigen, depigmentation was observed, but it was consistently weaker, more sporadic and started later. This autoimmunity is indicative of the inducement of an immune response to the self-proteins gp75 and TRP-2.

These tests were repeated with three independent mutant pools of 2,500 clones of mgp75 and four three independent mutant pools of 2,500 clones of mTRP-2. Every pool elicited both tumor immunity and autoimmune depigmentation.

Example 4

To show applicability of the present approach in multiple MHC alleles, mice from three different H-2 congenic strains (B10.A-H2a, B10.BR-H2k, B10.PL-H2u) were immunized with either of the following DNA preparations: unmutated mgp75, human gp75, and two independent pools of mutated mgp75 (2500 clones each). In a separate experiment, B10.BR-H2k mice were immunized with either unmutated mTRP-2, human TRP-2 or two independent pools of mutated mTRP-2 (2500 clones each). Autoimmune depigmentation was monitored in these mice as a measure of immunization efficiency. The fraction of depigmented mice over the total of each group is scored in Table 3. These results show that immunization with mutant DNA vaccine pools can immunized multiple

genetic backgrounds, suggesting that outbred populations such as humans will respond immunologically to this vaccine approach.

| Table 3 | | | |
|---|---|---|---|
| | H2a | H2k | H2u |
| wt mgp 75 | 0/7 | 1/10 | 0/7 |
| wt mTRP-2 | | 1/7 | |
| hgp 75 | 0/9 | 1/10 | 0/8 |
| hTRP-2 | | 0/8 | |
| mut mgp75 pool 1 | 1/10 | 4/10 | 4/9 |
| mut mTRP-2 pool 1 | | 3/10 | |
| mut mgp75 pool 2 | 0/7 | 3/10 | 7/9 |
| mut mTRP-2 pool 2 | | 6/10 | |

Example 5

BALB/c mice were immunized with a pool of plasmid DNA containing mutagenized PSMA. Antibody responses were measured by ELISA assays using diluted mouse sera against purified recombinant mouse and human PSMA made by expression of recombinant baculovirus in insect cells. Of ten mice tested, 10 has a positive response to human PSMA. Of another ten mice tested, 2 had a positive response to mouse PSMA.

Claims

1. A method for inducing an immune response to a target antigen in a subject comprising administering to the subject a vaccine composition in an amount sufficient to induce an immune response to the target antigen, wherein the vaccine composition comprises a plurality of vaccine molecule species corresponding to a plurality of mutant forms of the target antigen.

2. The method of claim 1, wherein the vaccine composition contains at least 50 vaccine molecule species.

3. The method of claim 1, wherein the vaccine composition contains at least 500 vaccine molecule species.

4. The method of any of claims 1-3, wherein the vaccine molecule species are nucleic acids, and the nucleic acids are expressed by the subject after administration.

5. The method of any of claims 1-3, wherein the vaccine molecule species are peptides.

6. A method for creating a nucleic acid vaccine composition for inducing an immune response to a target antigen, comprising the steps of:

(a) obtaining a starting sequence corresponding to a wild-type or mutant sequence of the target antigen; and

(b) randomly introducing mutations into the starting sequence to generate a mixture containing a plurality of nucleic acid sequences encoding mutant forms of the target antigen;

(c) packaging the mixture into dosage unit form for administration as a vaccine.

7. The method of claim 6, wherein the mixture contains at least 50 species of nucleic acid sequences.

8. The method of claim 6, wherein the mixture contains at least 500 species of nucleic acid sequences.

9. A method for creating a peptide vaccine composition for inducing an immune response to a target antigen, comprising the steps of:

(a) obtaining a starting sequence corresponding to a wild-type or mutant sequence of the target antigen; and

(b) randomly introducing mutations into the starting sequence to generate a nucleic acid mixture containing a plurality of nucleic acid sequences encoding mutant forms of the target antigen;

(c) expressing the plurality of nucleic acids sequences in the mixture to produce a peptide mixture; and

(d) packaging the peptide mixture into dosage unit form for administration as a vaccine.

10. The method of claim 9, wherein the nucleic acid mixture contains at least 50 species of nucleic acid sequences.

11. The method of claim 9, wherein the nucleic acid mixture contains at least 500 species of nucleic acid sequences.

12. A vaccine composition for inducing an immune response to a target antigen comprising carrier particles having coated thereon a mixture comprising a plurality of nucleic acid species encoding a plurality of mutant forms of the target antigen.

13. A therapeutic administration unit comprising:

(a) a hollow tubular carrier having an interior lumen; and

(b) vaccine composition for inducing an immune response to a target antigen comprising carrier particles (i.e. gold) having coated thereon a mixture comprising a plurality of nucleic acid species encoding a plurality of mutant forms of the target antigen disposed within the lumen.

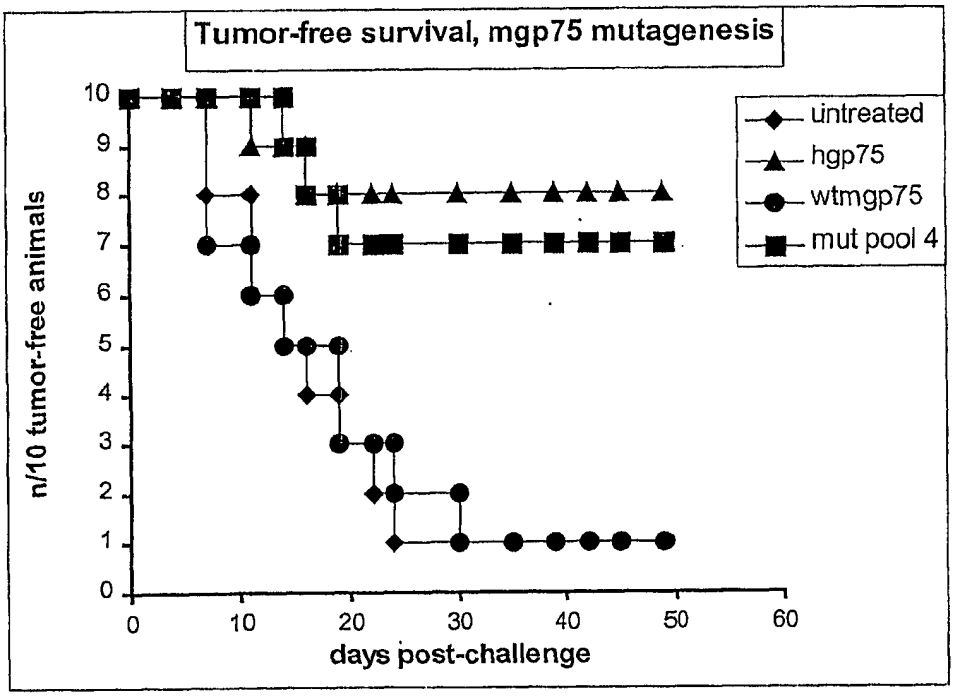
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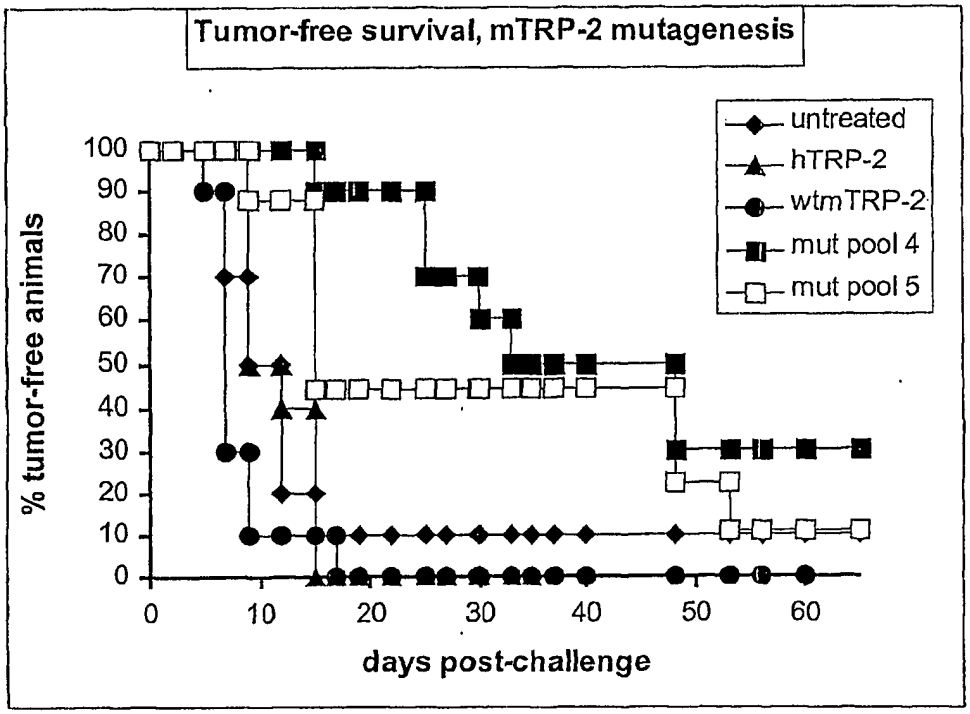
Fig. 1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mut pool 5
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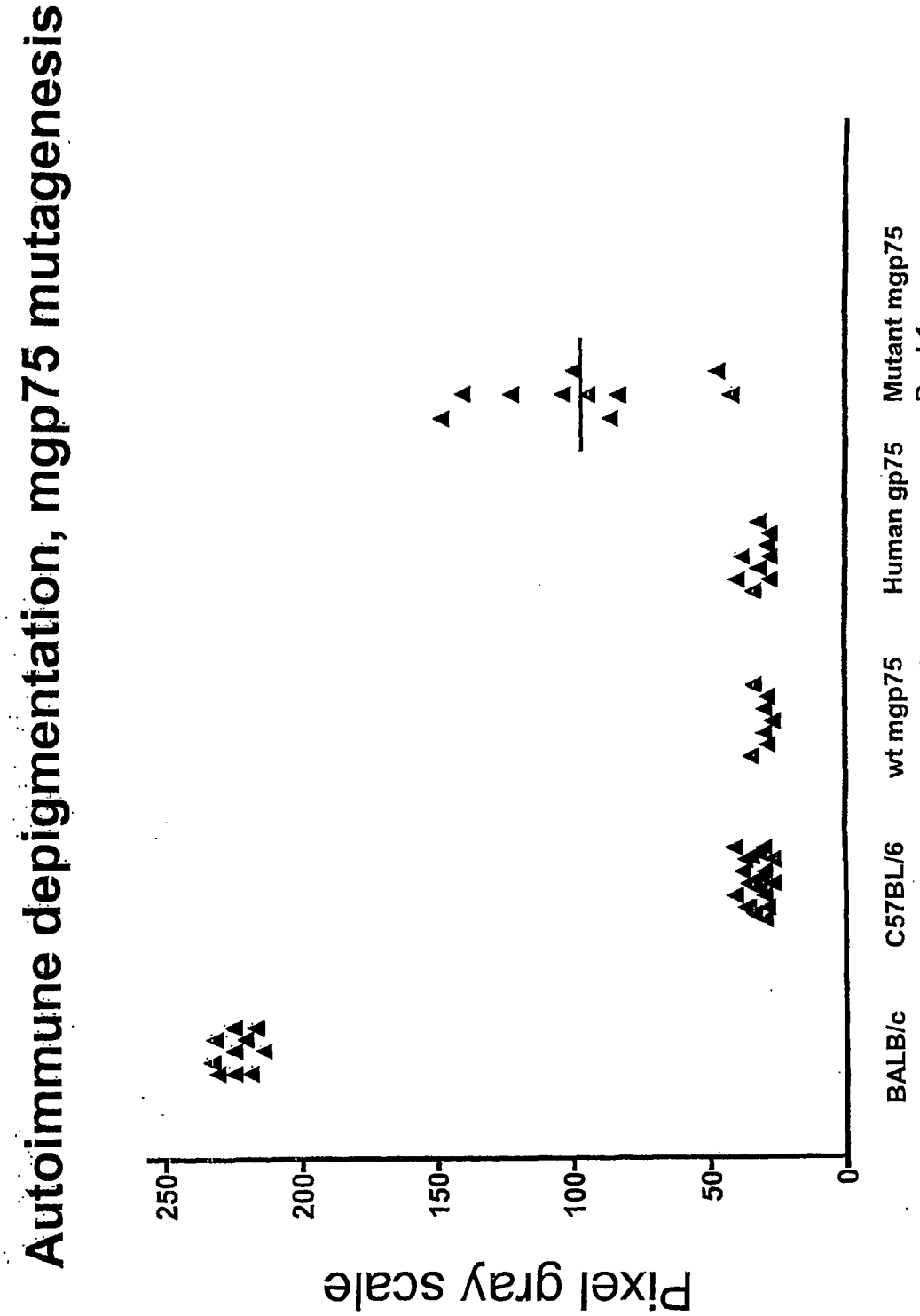
Fig. 2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Pool 1

Fig. 3

SEQUENCE LISTING

<110> Sloan-Kettering Institute For Cancer Research

Engelhorn, Manuel

Houghton, Alan

<120> Method and Composition for Immunization Using Mixed Pools of Mutated Nucleic Acids or Peptides

<130> MSK.P-044WO

<150> US 60/223,165

<151> 2000-08-07

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/24286

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 39/00, 38/04, 31/7105, 31/711; C12N 15/09, 15/01, 15/00

US CL : 514/2, 44; 424/184.1; 435/69.3, 440

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 44; 424/184.1; 435/69.3, 440

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 2.0, MEDICINE/BIOTECH (compendiumn databases on DIALOG) search terms: inventor names, vaccin?, nucle?, mutant, mutated, antigen, phage, recombinant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | DYALL et al. Heteroclitic Immunization Induces Tumor Immunity. J. Exp. Medicine. 02 November 1998, Vol. 188, No. 9, pages 1553-1561, see entire document. | 1-13 |
| Y | US 5,976,862 A (KAUFFMAN et al.) 02 November 1999, see entire document. | 1-13 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
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